**Supplemental Materials**

**Both Immune Priming and Egg-Adapted Changes in Vaccine Influence Antibody Responses to Circulating A(H1N1)pdm09 Viruses Following Influenza Vaccination in Adults**

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**Materials and Methods**

**Genetic Analysis**

HA segments of the viruses used in this study were sequenced in house. A complete HA1 alignment of amino acid sequences was made using BioEdit program with ClustalW method.

**HI assays**

HI assays were performed as previously described [1]. One part of sera was first treated with 3 parts of receptor destroying enzyme (RDE) at 37oC for 18-20 hours, followed by heat inactivation at 56oC for 30 min. Then sera were pre-diluted at 1:10 by adding 6 part of PBS. Before HI assays, sera were tested for non-specific agglutinins and adsorbed with packed turkey red blood cells (TRBCs) at 4oC for 30 min as needed, then centrifuged at 400g at 4oC for 5 min to spin down TRBCs.

For HI assays, sera were serially twofold diluted and incubated with 4 HA units/25 µl of virus for 30 min at room temperature. Fifty µl of TRBCs solution (0.5%) were then added to the wells and incubated for 30 min at room temperature before reading. HI titers were defined as the reciprocal of the last dilution of serum that completely inhibited hemagglutination. Antibody titers less than 10 were reported as 5 for calculation purposes.

**MN assays**

MN assays were performed as previously described [1]. Human sera were heat inactivated at 56°C for 30 minutes and pre-diluted at 1:10. Two-fold serially diluted sera were incubated with one hundred 50% tissue culture infection dose (TCID50) of influenza viruses and incubated at 37°C 5% CO2 for 1 hour. The virus-sera mixture was used to infect 1.5 × 104/well Madin-Darby Canine Kidney cells, and incubated for 18-20 hours at 37°C with 5% CO2. After cold acetone fixation, the presence of viral protein was quantified by the enzyme-linked immunosorbent assay using monoclonal antibodies specific to the nucleoproteins of the influenza A viruses. MN titers were defined as the reciprocal of the highest dilution of serum that gave 50% neutralization. Antibody titers <10 were reported as 5 for calculation purposes.

**Serum Adsorption Assay**

Briefly, 50 µl of serum sample was incubated with 180 µl 104.5~105 HAU of purified whole A(H1N1)pdm09 viruses, representative pre-2009 historic A(H1N1) viruses, or PBS control respectively for 2.5 hours at 4oC followed by ultracentrifugation at 168,941g for 30 min to remove virus-antibody complexes. To remove residue virus, sera were incubated with 100 µl packed TRBCs at 4oC for 30 min, then centrifuged at 400g at 4oC for 5 min to spin down virus-TRBCs complexes. Adsorbed sera were treated with RDE at 37oC overnight and heat inactivated at 56oC for 30 min, then kept in -20 oC until used. Adsorbed sera were tested by HI assay against the absorbent viruses to evaluate the reduction of antibody titers. Existence of dominant cross-reactive (CR) antibody among the testing viruses and the absorbent viruses was considered if adsorption of a serum with one or more heterologous viruses resulted in ≥ 4-fold reduction of HI antibodytiters to homologous virus compared to adsorption of the same serum with PBS control.

**Reverse genetically engineered viruses**

**Cells.** 293T human embryonic kidney (HEK293T) cells were obtained from the American Type Culture Collection (ATCC) and were maintained in Dulbecco Modified Eagle Medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen).

**Plasmids and recombinant DNA manipulations.** Recombinant DNA manipulations were essentially as described in Sambrook et al. [2] with minor modifications. One Shot Top 10 (Invitrogen, Carlsbad, CA) was used as the bacterial host. Restriction enzymes and T4 DNA ligase (New England BioLabs, Beverly, MA) were employed as recommended by the manufacturers. The viral hemagglutinin (HA) from A/California/08/2009(H1N1pdm09) and neuramindase (NA) from A/California/04/2009(H1N1pdm09) (NA sequence is identical to A/California/08/2009(H1N1pdm09)) were cloned into a dual-promoter plasmid vector under control of the human polymerase I promoter and the mouse RNA polymerase I terminator [3]. To generate HA mutants, three-round, asymmetric PCR amplification strategy was employed as described previously [4]. HA mutants were generated using Quickchange Lightening Site-directed Mutagenesis kit (Agilent Technologies, Santa Clara, CA) and mutagenesis primers.

**Derivation of influenza viruses by reverse genetics.** Reassortant viruses were generated from plasmids by reverse genetics methods [3]. Reverse genetics plasmids encoding the HA or HA mutants and NA genes were co-transfected with six plasmids expressing internal genes from A/Puerto Rico/8/1934 (PR8) into 293T cells using Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. 0.3 μg/ml TPCK-treated trypsin (Sigma-Aldrich, St. Louis, MO) was added to culture supernatants two days post-transfection and after an additional 24 hours transfected cells were resuspended in culture media and inoculated into 9-11 day-old embryonated hen eggs (Charles Rivers Laboratories, North Franklin, CT). Allantoic fluid was harvested two days after inoculation and the presence of virus was revealed by agglutination of turkey red blood cells. The serially diluted virus was inoculated into eggs to obtain C1E2 working stocks of reverse genetics-derived reassortant virus and stored at −80 °C.

**Genomic sequence analysis.** Total RNA was extracted from allantoic fluid using the QIAmp Viral RNA Minikit (Qiagen; Valencia, CA) and reverse transcribed to viral cDNA and amplified using a one-step reaction system (OneStep RT-PCR Kit, Qiagen) with sequence-specific primers. RT-PCR products were purified by ExoSAP-IT system (Affymetrix/USB, Cleveland, OH). Sanger sequencing of the cDNA was performed using the BigDye Terminator v3.1 Cycle Sequencing kit (Life Technologies Corporation, Grand Island, New York). The sequencing extension products were purified using the BigDye Xterminator Purification kit (Life Technologies Corporation) and analyzed using an ABI 3730 DNA Analyzer (Applied Biosystems, Grand Island, NY, USA) according to the instructions of the manufacturer.

**Supplemental Figure 1. Epidemiological history and antigenic grouping of the seasonal human influenza A(H1N1) viruses from 1977 to present.** (A) The representative seasonal A(H1N1) vaccine strain-like viruses are summarized for 1977-1978 to 2016-2017 seasons. Viruses are color-coded accordingly based on the phylogenetic analysis of HA sequences, antigenic relatedness illustrated with primary infection ferret antisera and human immune sera.The span of the colored frame for each virus corresponds the seasons when this virus was circulating and isolated. There are four major antigenic groups designated so far. Note that for the four viruses in the group with a deletion in HA1 amino acid position 130 (130-deletion), they show antigenic divergence based on ferret antisera and human serology data, though they are still antigenically more related compared with the remaining three groups. They are grouped together primarily based on the common feature of 130-deletion in the HA head domain. (B) Percentages of seasonal H1N1 isolates among all the influenza A (H3N2 & H1N1) and B isolates are summarized for 1977-1978 to 2016-2017 seasons [5-79]. Bars represent the proportion for each season and are color-coded corresponding to the antigenic groups in (A). The black bar for 1999-2000 season indicates a mix of A/Bayern/7/1995, A/Beijing/262/1995 and A/New Caledonia/20/1999-like viruses isolated in that season. Dashed line denotes 20% which is generally an indicator for predominate circulation.

**Supplemental Figure 2. Shared HA 163K epitope correlated USSR/77-priming with reduced HI antibody reactivity to 163Q H1N1pdm viruses.** Shown here are individual HI antibody titers to A(H1N1)pdm09 (A) and prior-2009 sH1N1 viruses (B) from the 29 USSR/77-primed persons carrying 163K-specific antibody. Open circles and black solid circles represent respectively pre- and post-vaccination titers. Dashed line indicates HI titer of 40. Horizontal and error bars stand for GMT+95% confidence interval (CI). Amino acids for HA1 125, 127, 163 and 223 sites are noted below for each virus. \* The NXT motif for HA1 125-127 suggests a potential glycosylation at amino acid position 125. ¶ Number of cases that seroconverted to the corresponding testing sH1N1 virus.

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